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Original Article

Ecodye & Bio Cleanser: Innovating Sustainability With Natural Fabric Dyes And Microbial Decolorizers

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ABSTRACT

The textile industry is at a turning point due to the increasing demand for eco-friendly products. Natural fabric dyes and organic decolorizers offer promising solutions for sustainable textile production. Plant materials such as Bougainvillea, Balsamina, Clitoria ternatea, Cestrum nocturnum, Mirabilis jalapa, and Red Malabar Spinach seeds are used as natural sources of fabric dyes. Cream of tartar is employed as a mordant to enhance dye retention, thereby improving the durability and sustainability of dyed fabrics. After mordanting, the fabrics are immersed directly in the extracted pigments for 24 hours. Microbial decolorizers provide an environmentally friendly approach to removing dyes from fabrics. Bacillus species produce enzymes such as laccases, peroxidases, and azoreductases, which break down both synthetic and natural dyes, enabling efficient decolorization without the use of harsh chemicals. These bacteria effectively decolorize natural dyes through enzymatic reactions. Similarly, microorganisms such as Aspergillus niger and yeast (Saccharomyces cerevisiae) possess enzymatic activities that facilitate the decolorization of naturally dyed fabrics. Activated charcoal is another effective decolorizing agent due to its excellent adsorption capacity and reusability. These eco-friendly alternatives offer sustainable solutions for reducing the environmental impact of textile dyeing processes. Various shades of dyed fabrics were obtained using different plant sources. For example, Night Jasmine yielded a yellow color, Balsamina flowers produced a yellowish-brown hue, Red Malabar Spinach generated a magenta color, Bougainvillea flowers resulted in a pink shade, and Clitoria ternatea produced a blue color on both cotton and polyester fabrics. In addition, Curcuma longa (turmeric) combined with baking soda produced an orange color, while Mirabilis jalapa was used to dye fabrics grey. This study investigates the decolorization of various naturally dyed fabrics using four different methods: bacteria (Bacillus species), yeast, fungus (Aspergillus niger), and activated charcoal. The results demonstrated that all four methods were successful in decolorizing the fabrics. Bacterial treatment required 7 days, yeast treatment required 5 days, whereas fungal treatment and activated charcoal required 9 days to achieve decolorization. The findings of this study provide valuable insights into sustainable dye removal techniques for textile applications.

Keywords: Natural fabric dye, Bougainvillea, Balsamina, Clitoria ternatea, Cestrum nocturnum, Mirabilis jalapa, Basella rubra.

INTRODUCTION

Over time, people have increasingly shifted toward natural products due to changing consumer preferences and growing awareness of health and environmental concerns. Ongoing research and investigations have made consumers more apprehensive about the potential health risks associated with synthetic or chemical components used in various products. As a result, they are seeking alternatives that are perceived as safer, healthier, and more environmentally sustainable. Natural fabric dyes are gaining popularity for both environmental and health-related reasons. The production of synthetic dyes is largely dependent on petrochemical sources, and some synthetic colorants contain carcinogenic amines (Hunger, 2003). Consequently, the use of natural dyes is increasing due to their non-toxic, non-carcinogenic, eco-friendly, and biodegradable nature. They also reduce chemical exposure, are easily accessible, and can be obtained from renewable and sustainable resources such as flowers and other plant materials. In contrast, synthetic dyes are often non-biodegradable and tend to accumulate in the environment, causing significant ecological damage. It has been estimated that large quantities of synthetic dyes are used annually

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worldwide. The discharge of synthetic dyes can lead to environmental pollution, skin disorders, and various health hazards affecting humans and other living organisms. Research on natural dyes encompasses a broad range of topics, including the identification of new dye sources, improvement of dyeing techniques, and exploration of their applications in different industries. Beyond their coloring properties, many natural colorants also possess antibacterial and antifungal activities. India is one of the world's richest biodiversity regions, ranking 11th globally in terms of floral diversity. The country is home to approximately 490,000 plant species, making the plant kingdom a valuable reservoir of natural products (Neha Grover et al., 2011). Flowers, in particular, serve as important sources of natural dyes. Floral pigments impart a wide range of attractive hues and pleasant aromas to fabrics. They are rich in tannins, which contribute to the production of various shades, including red, pink, brown, and yellow. Furthermore, the cultivation, harvesting, and processing of dye-yielding plants create economic opportunities for local communities, thereby supporting sustainable livelihoods and promoting environmentally responsible textile practices.

***ClitoriaTernatea*: -**substantially belongs to the family Fabaceae. Due to its pharmacological exercise, it's extensively used as an antimicrobial, antipyretic, anti-inflammatory, analgesic, and numerous further. The flower can be used to stain natural threads and is used by traditional societies in Asia to do so. Blue single petal floral size "8 – 16″ altitudinous (including pot).

***RedMalabarSpinach*: -** It belongs to the family Primulaceae. It's gathered in veritably large quantities due to its 75 ayurvedic medications. It's rich in anthelmintic, carminative, antibacterial, antibiotic, hypoglycemic, and anti-fertility properties.

***CurcumaLonga* (Turmeric) + baking soda: -**A cremating brew. It belongs to the family Zingiberaceae. Turmeric is rich in anti-inflammatory, antimicrobial, antioxidant, and anti-neoplastic properties. It also has curcumin which may be used medically to treat a variety of dermatologic conditions. Pre-dyestudy revealed the following Turmeric does notneed a caustic to set the stain. It's also what is called a fugitivestain- the chemical nature of fugitive colorants means they are kind of semi-permanent.

***Bougainvillea*: -** It belongs to the family Nyctaginaceae. It's rich in anticancer, antihepatotoxic, anti-inflammatory, antihyperlipidemic, antimicrobial, antioxidant, and anti-ulcer properties. Flowers are introduced in axillary clusters of three, each flower with a grandiloquent, red, pink, or orange bract beneath. The calyx is tubular with 5 lobes roughly 2 cm long. The tube is the same color as the bracts, with white lobes roughly 3 mm long and 5- 10 stamens.

***Balsamina*: -** It belongs to the family Balsaminaceae. The floral grows up to a height of 20 to 75 cm. They've mug-shaped, various flowers with four to five petals and grow up to one to three altitudes in size. In Southeast Asia, it was also used in making stains. For exemplar, in Nepal, balsam leaves were crushed to color fingernails on the day of Shravan Sankranti.

***CestrumNocturnum* (Night Jasmine): -**It belongs to the family Oleaceae. The flowers are ambrosial, with a five- to eight-lobed white corolla with an orange-red center; they're produced in clusters of two to seven together, with individual flowers opening at dusk and finishing at dawn. The leaves have been used in Ayurvedic medication and Homoeopathy. like antioxidant, antibacterial, antifungal, anticancer, antimalarial, antiepileptic, cytotoxic, analgesic, anti-inflammatory, anti-HIV, hepatoprotective, antipyretic, and injury recovery properties.

***MirabilisJalapa*: -** Also known as the four o'clock flower. It belongs to the family Nyctaginaceae. The flowers generally open late evening or dusk (between 4 and 8 o'clock). The flowers aren't formed from petals – rather they're a colored difference of the calyx. The flowers are pipe-shaped and penta-lobed, they've no mug (replaced by bracteal leaves) but are made of a corolla. It has a high capability for bioremediation, reduces inflammation, and numerous further. Used in making stains in the fabric diligence.

Decolorization by Bacteria, Yeast, Fungus (*AspergillusNiger*), and activated charcoal

Dyes usually have complex aromatic structures which make them more stable and difficult to degrade. There are more than 40,000 dyes and pigments with some 7000 different chemical structures, out of which more

than 3500 dyes are in practical use. Synthetic dyes are classified as azo dyes, nitroso dyes, triphenylmethane dyes, xanthene dyes, and anthraquinone dyes (Shenai, 1994). The existing methods that are used in the degradation process are found to be highly expensive and due to the production of chemical sludge, it is very harmful to the flora and fauna including human beings. Microbial degradation and decolorization of dyes are seen as a cost-effective method for removing this pollution from the environment (Banat et al.,1996; Pearce et al.,2003). Several bacterial, fungal, and algal species can adsorb and/or break down azo dyes. (Stolz, 2001; Don Santoz, 2007). Moreover, bacterial decolorization is normally faster than fungal systems concerning the decolorization and mineralization of azo dyes (Banat et al., 1996). Numerous bacterial species including Bacillus, Pseudomonas, Enterobacter, Halobacter, and Aeromonas have been decolorized and detoxified by a wide range of azo dyes compared to phenylamine, benzenediazonium chloride or phenol (Telke et al.,2008; Mendes t al.,2011; Feng et al.,2012).Activated carbon is a remarkable material that possesses a structurally homogeneous composition, boasts a high surface area, and demonstrates exceptional radiation stability. With its microporous structure, it has become a go-to solution for adsorption, catalysis, and catalyst support in various industrial processes. The effectiveness of activated carbon's adsorption properties largely depends on its particle size, porosity, ash content, degree of carbonization, and method of activation. The concentration of hydrogen ions, also known as pH, significantly impacts the dyes' ionization and the adsorbents' surface properties. The adsorption of the selected dyes decreases with an increase in pH, except for turmeric (containing 30% baking soda) on activated charcoal. The reason for this is that activated charcoal forms a positively charged surface. When the pH value is low, the negative charge on the surface of the charcoal decreases, increasing the positive charge on the surface, which, in turn, enhances the adsorption of the negatively charged adsorbate.

MATERIALS AND METHODS:

Collection of samples:

We collected the samples from various places to get the color pigments from various plant sources. The *Clitoria Ternatea* flowers were collected from Ramakrishna Mission Ashram, Satara Parisar, *Mirabilis Jalapa, Red Malabar Spinach, Balsamina* were collected from the Shivsena Pramukh Sriman Balasaheb Thakre Botanical Buddi Garden, Chaitanya Housing Society, Cidco Rd, Shivdatta Housing Society, N 8, Yashashri Colony N 8, *Bougainvillea* flower was collected from Prayas Biodiversity HotSpot, Nyay Nagar, *Curcuma Longa* (Turmeric) was collected from K.K Dawasaz, shahganj, Cestrum nocturnum (Night Jasmine) flower was collected from the Parijat Botanical Garden, sector N4 Cidco.

Other Glassware like Flasks, beakers, test tubes, glass rods, Petri plates, weighing balance, spatula, cream of tartar, burner, mortar, pestle, etc. were collected from the laboratory of the Biotechnology department of Deogiri College. All the flowers were collected from locations situated in Chhatrapatinagar, Sambhajinagar.

Materials used for the decolorization process -

Nutrient broth, Czepedox broth, nutrient Agar, Agar agar powder, activated charcoal, distilled water, Glucose, Dry yeast, and glassware like flasks, test tubes, 2 Petri plates, and weighing balance were collected from the laboratory of the Department of biotechnology, Deogiri College.

Figure 1: Collection of sample: Onion infected with black mold collected from the local vegetable market of Chhatrapati Sambhajinagar.

METHOD OF EXTRACTION OF DYES FROM THE FLOWERING PLANTS

The aqueous extraction method was used for the extraction of dyes from different parts of the flowering plants including seeds. In this method dyes from flowers were extracted by preparing an aqueous solution of flowers. Firstly, the flowers were exposed to the natural sunlight until and unless they become completely dry. Once it dried completely, they were crushed into fine powder manually in mortar and pestle. Then, they were soaked in water to get the pigments for 3-4 hours or sometimes overnight depending upon the flowers. The quantity of the powder to be put in the water was dependent upon the availability of the flower powder (the standard can be taken as 10 gm in 100 ml distilled water).Then they were boiled at (80-85 degrees) until a nice color was obtained and then filtered to remove the non-coloring particles. The Dye colors were then stored in the refrigerator for further use.

Exception: In the case of seeds of Red Malabar Spinach, seeds were crushed into a fine paste using a mortar and pestle. Once the fine paste was obtained it was mixed with water for some time and then filtered using filter paper.

For the preparation of orange dye, baking soda was added to the turmeric powder to create a color variation from yellow to orange. The process has been explained in detail in the below diagram of the Bougainvillea flower. The same process was carried out for the rest of the flowers used in the experiment:

BougainvilleMirabilis Jalapa, *Clitoria Ternatea*, *CestrumNocturnum*(Nightjasmine), *Balsamina.*

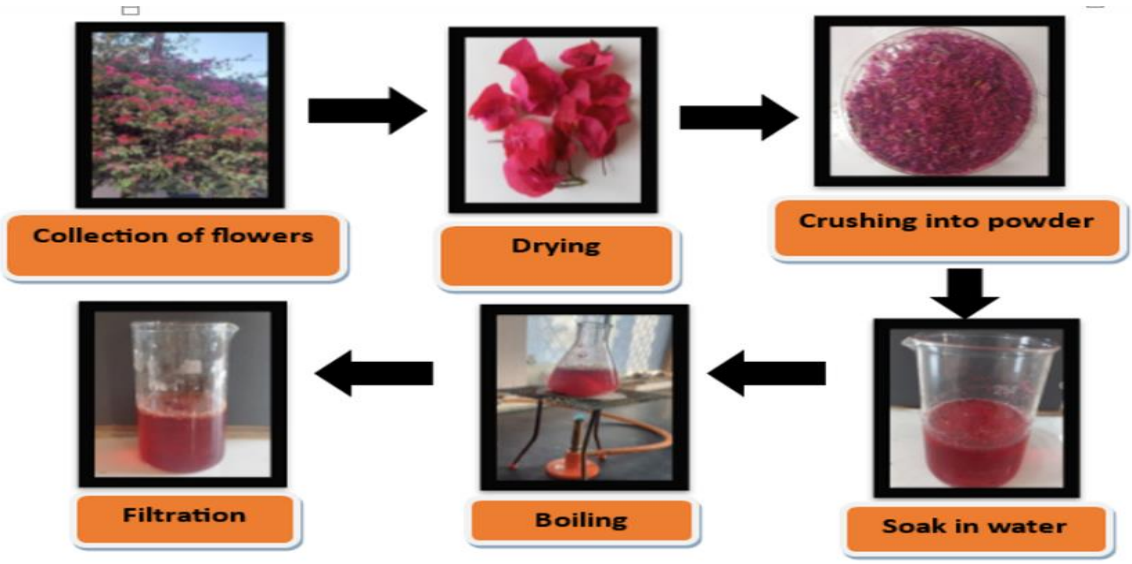


Fig 2: Extraction process of dye from Bougainvillea flower

For the extraction of dye from the seeds of Red Malabar Spinach, firstly the seeds were crushed into fine paste using filter paper. After the preparation of the fine paste, Paste was mixed with water and then filtered out.No boiling is required in the use of seeds.

Fig: 3 Crushing seeds of Red Malabar Spinach

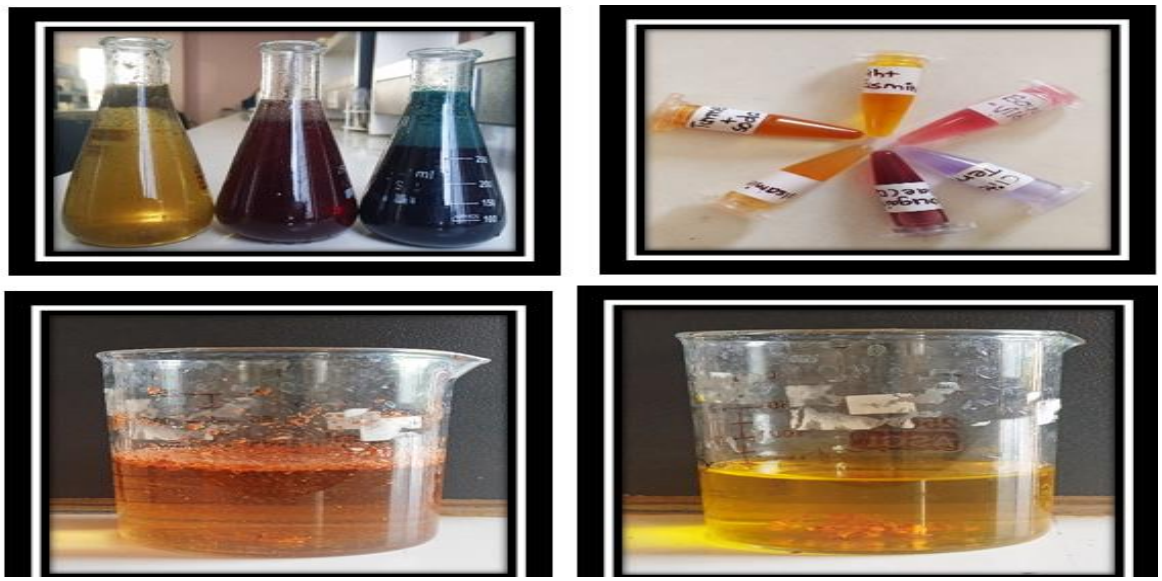


Fig: 4 All the extracted pigments from the flowers

Mordanting Of the Fabric:

Mordants: are chemicals in the form of metallic salts that are generally used to create an affinity between the fiber and the pigment. The mordant which was used for the mordanting was Cream of Tartar.

Cream of tartar: - Also known as tartaric acid, it is a byproduct left after the fermentation of grapes into wine.

It is used as 6% fiber weight for 100 g of fiber.It is used for woolen fibers but after experimenting with cotton and cotton clothes, but later it was observed that it is more efficient than vinegar and salt mordant.

Fig.6 Mordanting By Cream Of Tartar

Fig. 5 Mordanting By Salt

Process of Mordanting

First, fill the flask with tap water and make it lukewarm with the help of a burner. Add the standard amount of cream of tartar to the flask and allow it to dissolve completely. Then add the fabric to the solution for about half an hour. Make sure the fabric is covered completely in the solution. After half an hour, let the fabric cool completely and wash it under tap water. After that, the dye was added to the mordanting cloth and left for a few hours according to the dying fastness.

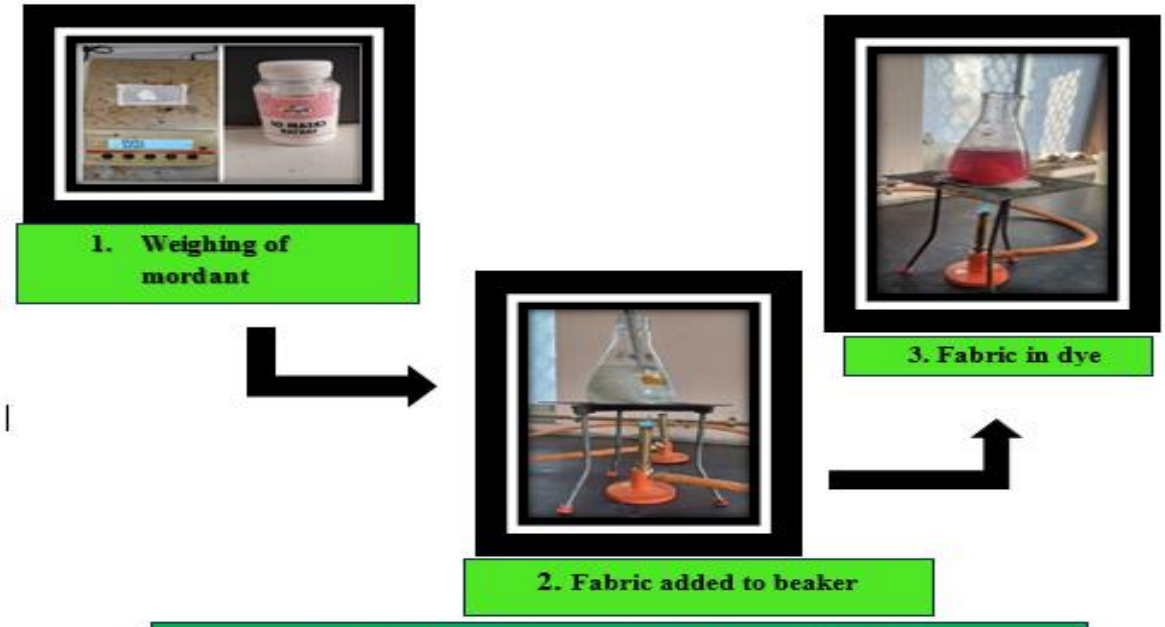
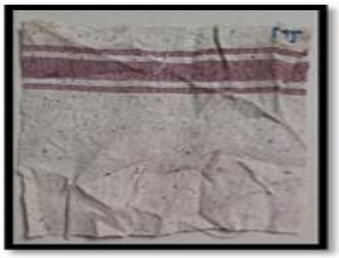


Fig.7: - Process of Mordanting

CestrumNocturnum
(Night jasmine)
Cotton
Polyester

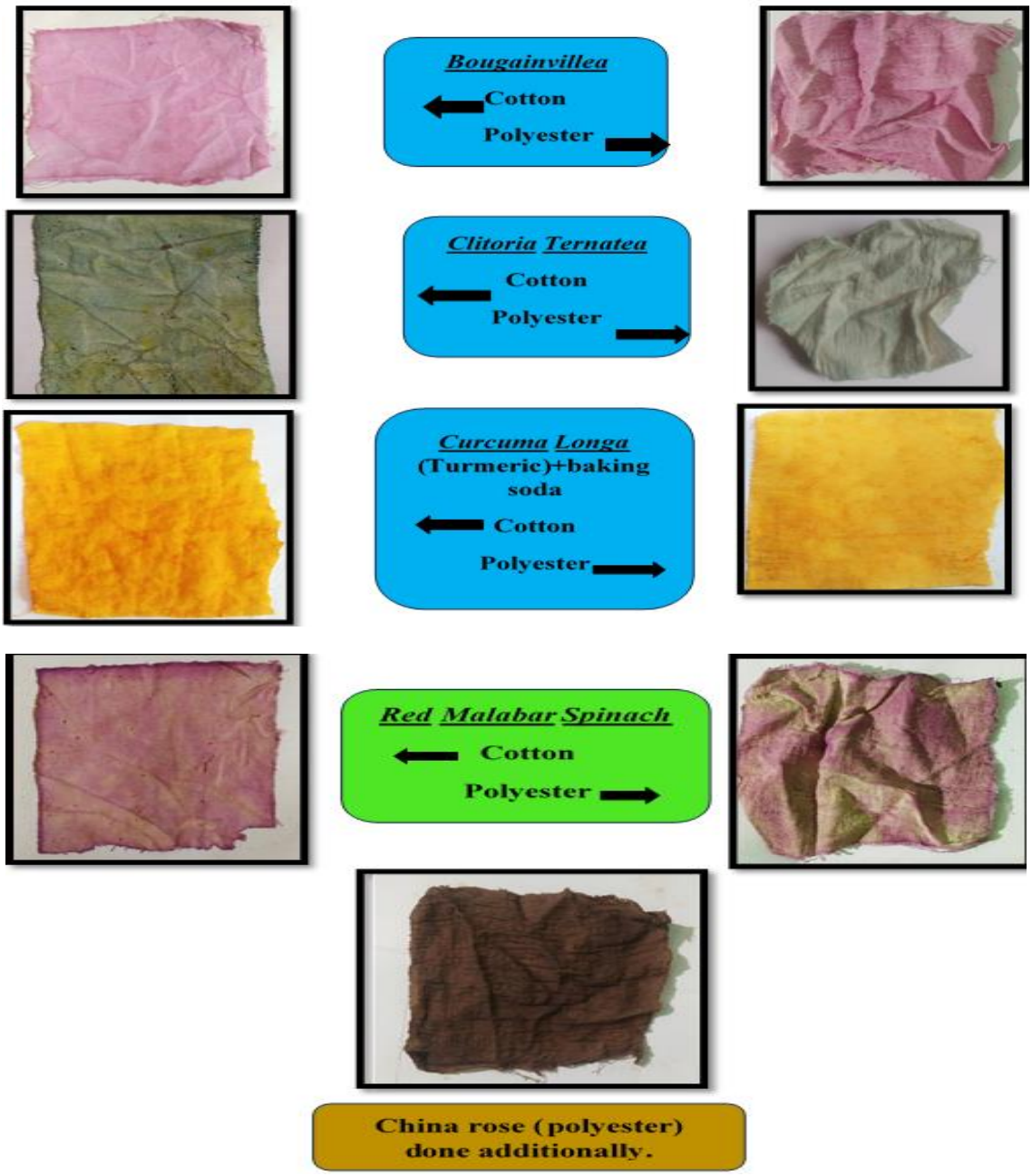
Balsamina
Cotton
Polyester



Mirabilis Jalapa
Cotton
Polyester

Cestrum Nocturnum
(Night jasmine)
Cotton
Polyester

Balsamina
Cotton
Polyester



Decolorization by the Bacteria, Yeast, Fungus and Activated Charcoal

Figure 8. Dyed cotton and polyester fabrics showing colors obtained from various natural dye sources.

Three methods are tested for the decolorization of fabric dyes such as activated charcoal, bacillus sp., yeast, and fungus (*AspergillusNiger*).

Preparation of the nutrient broth containing bacterial culture

Firstly, the nutrient agar was prepared.The volume of nutrient Agar required was calculated as per the standard volume mentioned on the bottle (28gm in 1000 ml distilled water.) and 1% of Agar agar powder was added to it. Thus, the media was prepared.After the preparation of the media, it was autoclaved at 121 degrees for 15 minutes. Media was poured into the Petri plate in the Laminar Air Flow under Sterile conditions and cooled down.After the media solidified, the loopful sample from 10^{-6} test tubes of soil serial dilution was taken and 4 quadrant streaking was done and incubated at 37 °C for 24 hours.After the incubation, the loopful of bacterial colonies were taken from a cultured plate and inoculated in the nutrient broth under Laminar Air Flow at sterile conditions and incubated overnight.After the incubation, an equal amount of volume was

distributed in each test tube containing small pieces of each dyed fabric.Then the test tubes were left for several days until the decolorization was observed in each test tube and the results were recorded.

Preparation of the yeast culture

Firstly, the dry yeast was taken from the laboratory and put in the glucose solution of standard concentration (1g in 100 ml).Incubate the solution for overnight at room temperature.After that, an equal amount of volume was distributed in each test tube containing small pieces of dyed fabric.

Preparation of the Czepedox Broth containing *Aspergillus Niger*

Firstly, the Czepedox broth was prepared. The volume of czepedox broth required was calculated as per the standard volume mentioned on the bottle (35.01g in 1000 ml distilled water.) and 1% of Agar agar powder was added according to it.After the preparation of the Czepedox media, it was autoclaved at 121 degrees for 15 minutes. After the media preparation, the loopful of black mold was picked from the onion and spotted on the czepedox plate. Incubated the plate for 5 days to get the *AspergillusNiger*.After the incubation, the loopful colony was inoculated in nutrient broth (prepared by the standard volume of 25g in 1000 ml of distilled water) and again incubated overnight.After overnight incubation, broth was distributed 20 ml in each test tube containing small pieces of dyed fabric.Then it was left for several days until the decolorization was observed and the results were recorded.

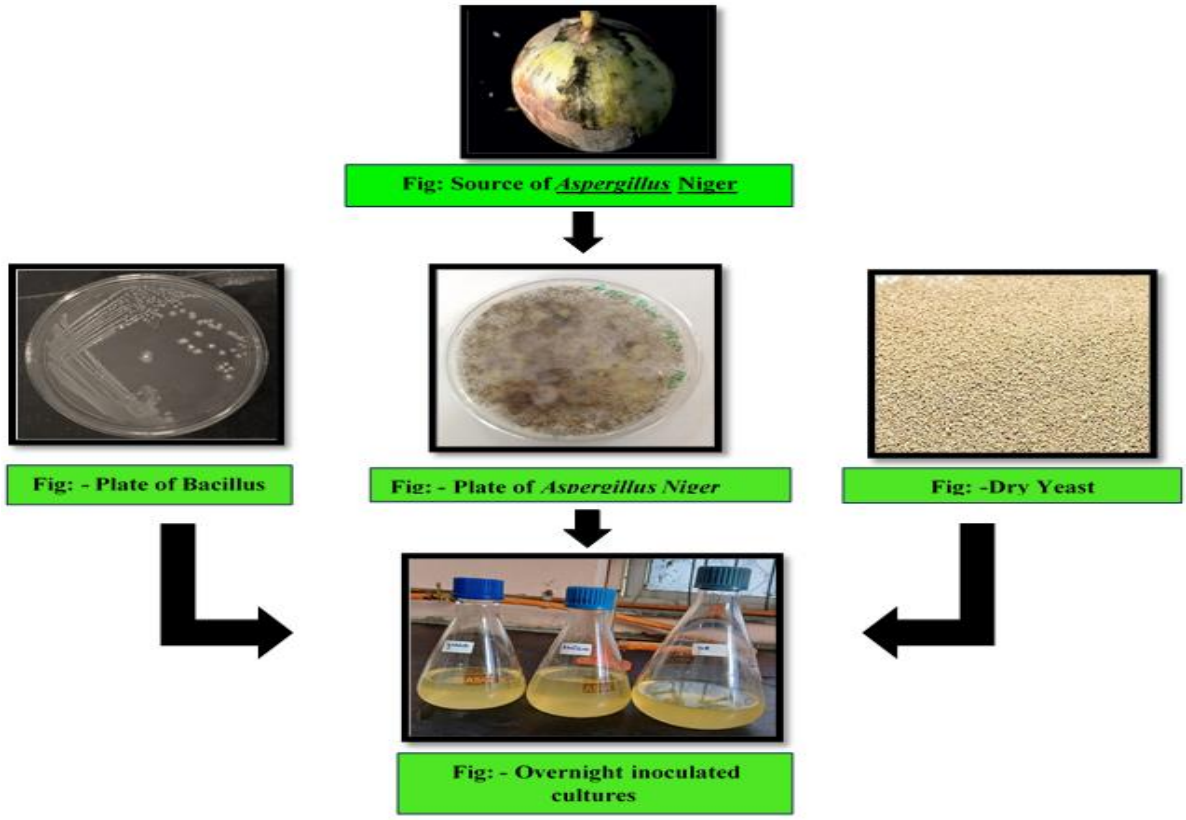


FIG 9: Inoculated Cultures of Bacillus, Fungus and Yeast

Preparation of Activated Charcoal Decolorizer

5 grams of activated charcoal was dissolved in 100 ml of distilled water mixed well and then filtered out filter paper to get water like clear solution of activated charcoal. Then colored cloth strips were dipped into it.

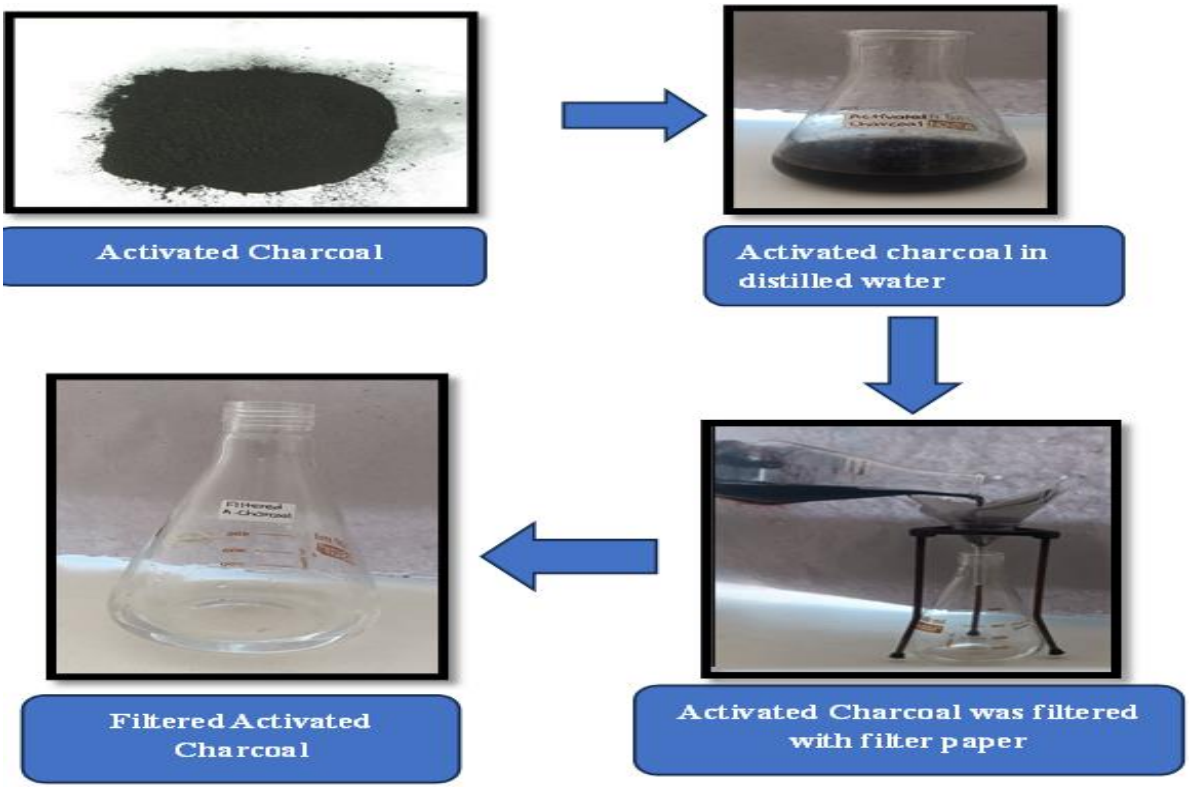


FIG 10: Activated charcoal decolorizer production process

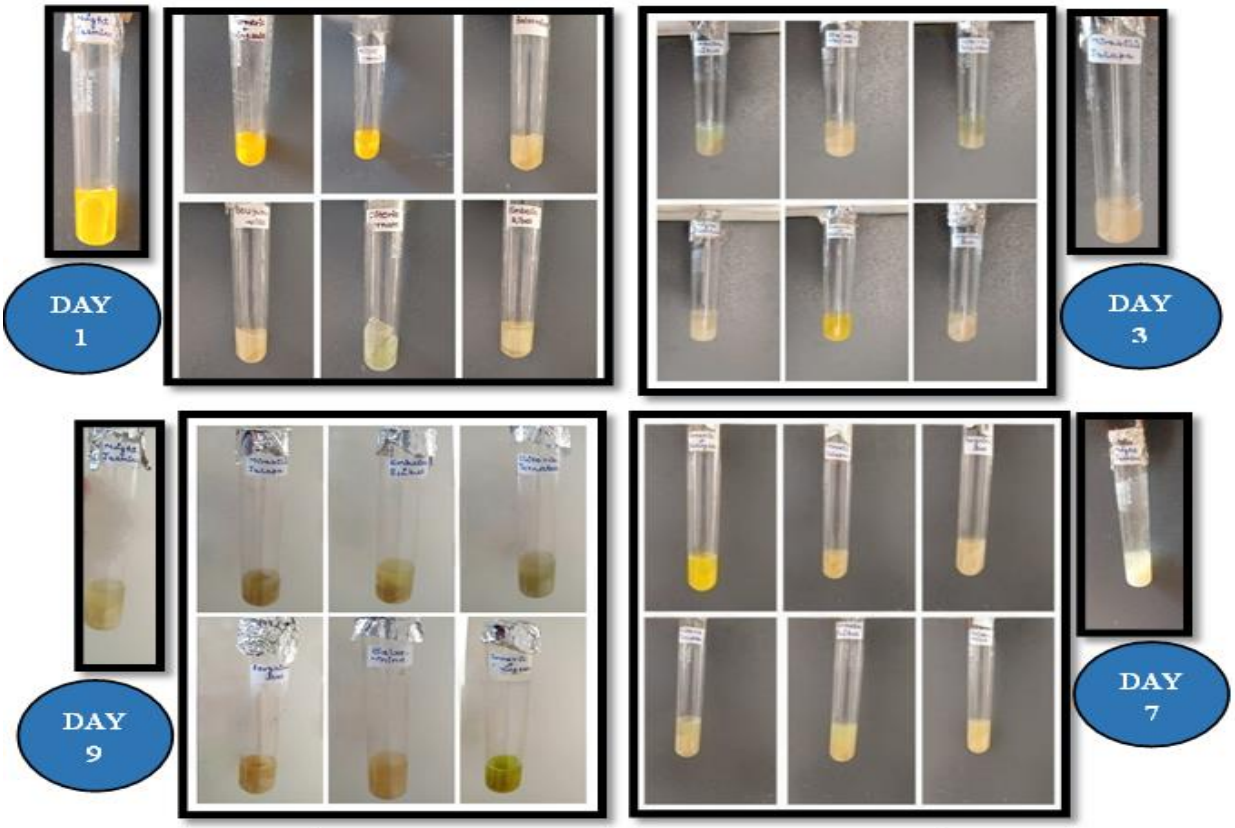


FIG 11: - Bacterial Decolorization

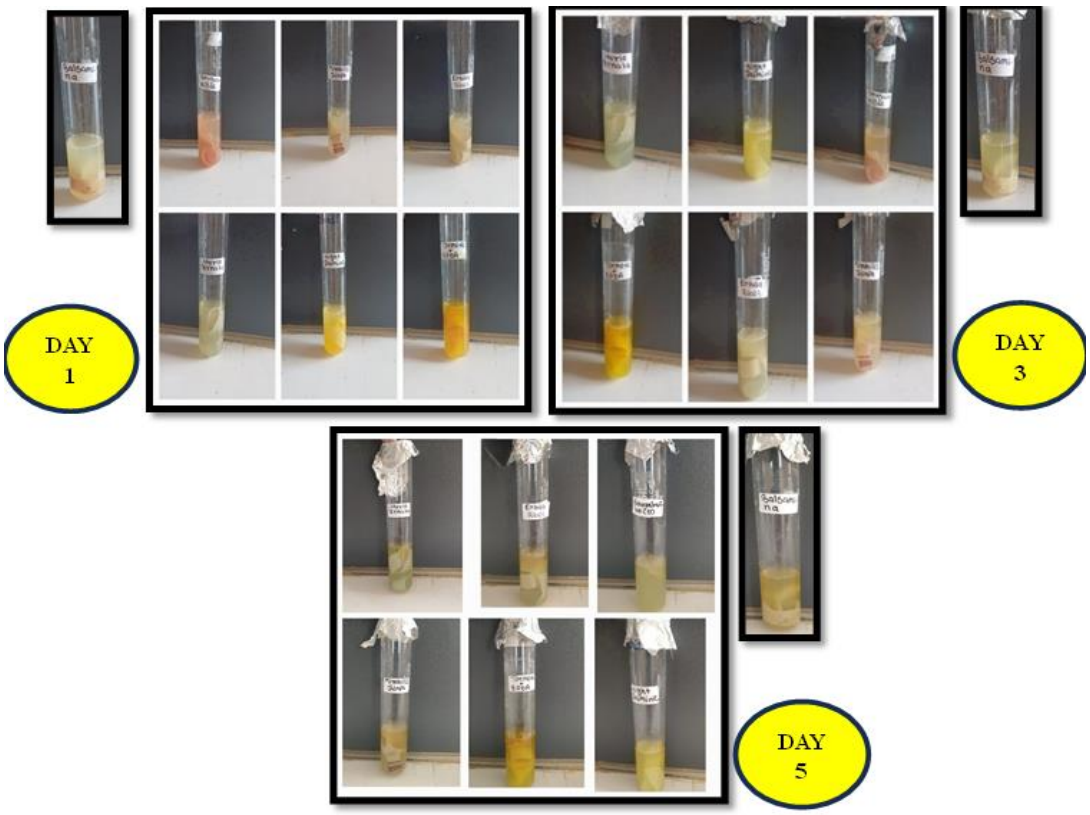


FIG 11: - Yeast Decolorization

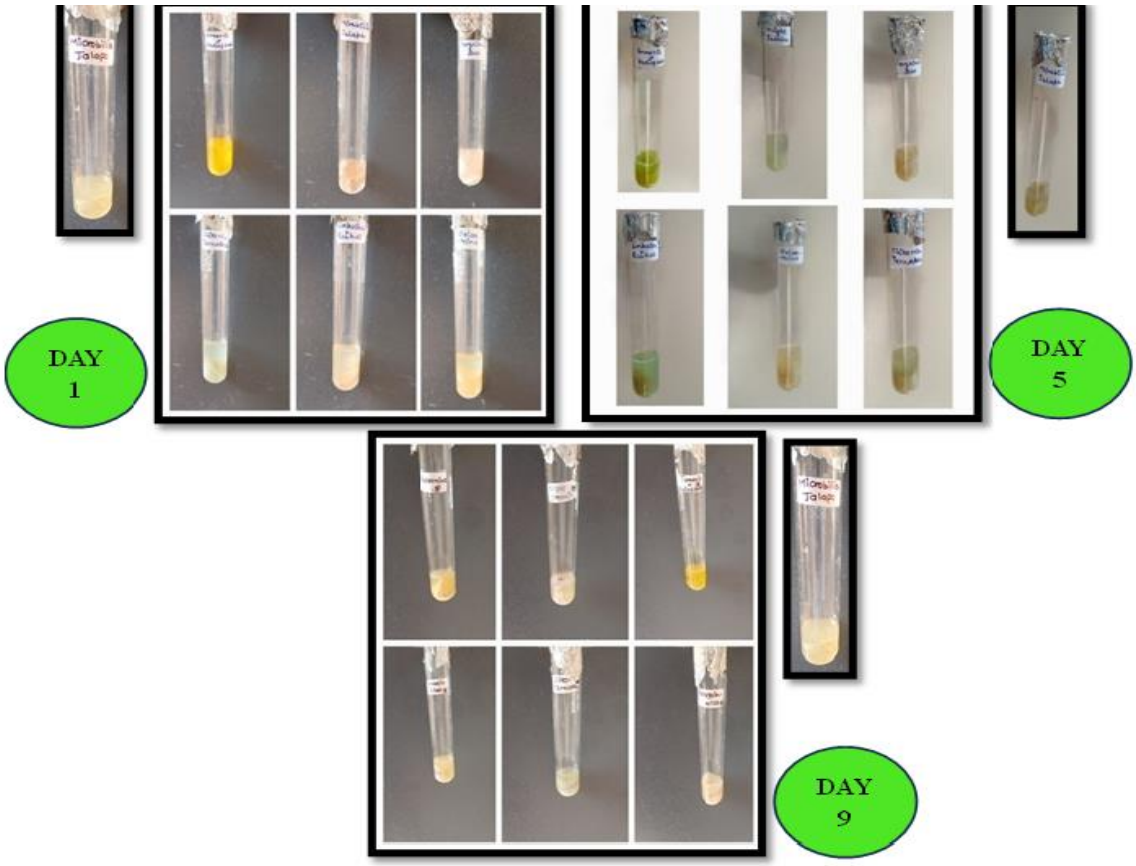


FIG 12: - Fungus Decolorization

RESULT:

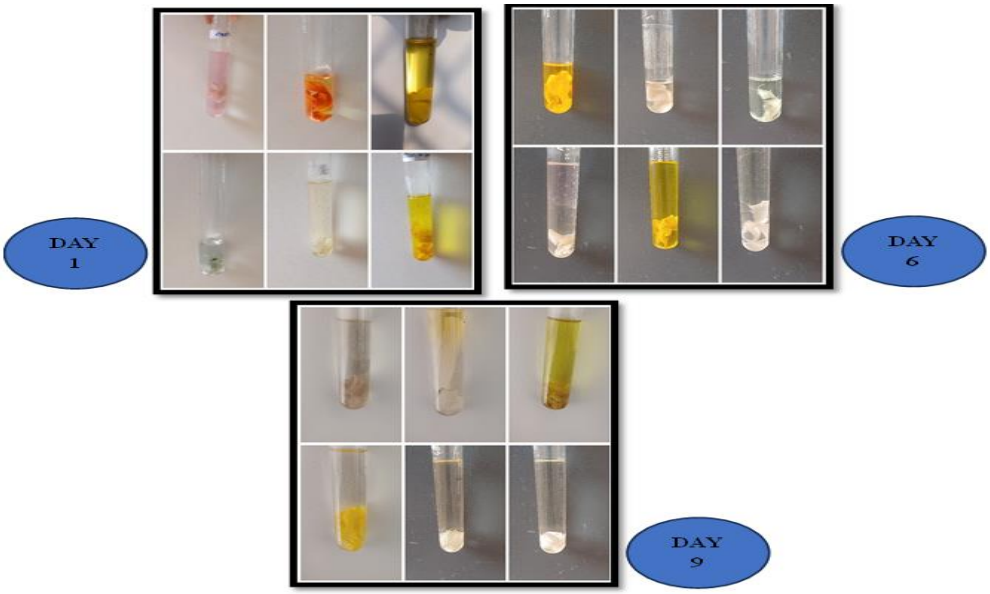


FIG 13: - Activated Charcoal Decolorization

FIG 14: Bacterial Decolorization

FIG 15: Yeast Decolorization

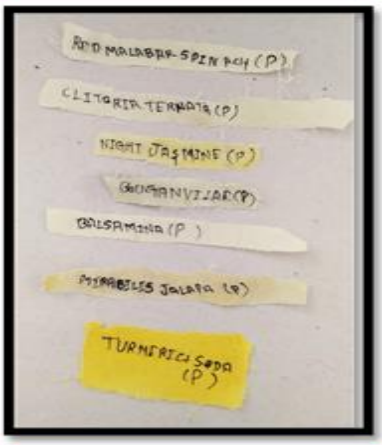


FIG 16: Fungus Decolorization

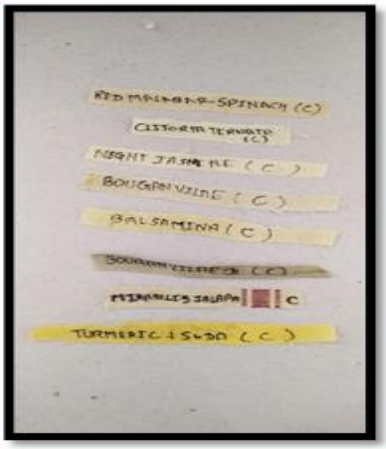


FIG 17: Activated Charcoal Decolorization

FIG 18: shows the decolorization of dye using four different methods.

These are strips of dyes that have been decolorized using various methods. Different time intervals were observed for different methods used. For instance, clothes that were dyed using the bacterial decolorization method took 7 days to show results, whereas those dyed using the yeast decolorization method took 5 days. Clothes dyed using the fungus decolorization method showed results after 9 days and those dyed using activated charcoal took 9 days as well.

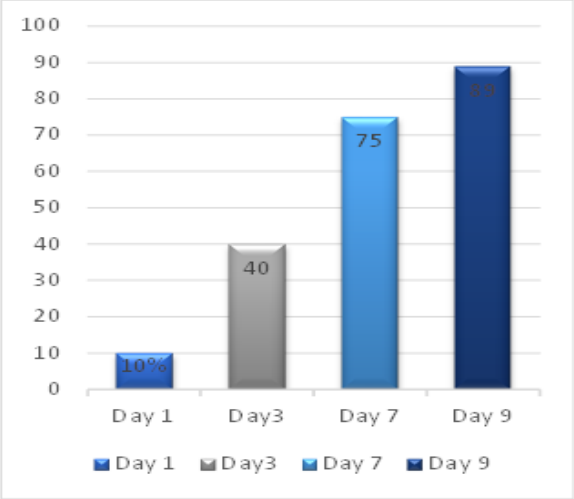


FIG 19: -Percentage of bacterial decolorization of dyes for a different interval of days

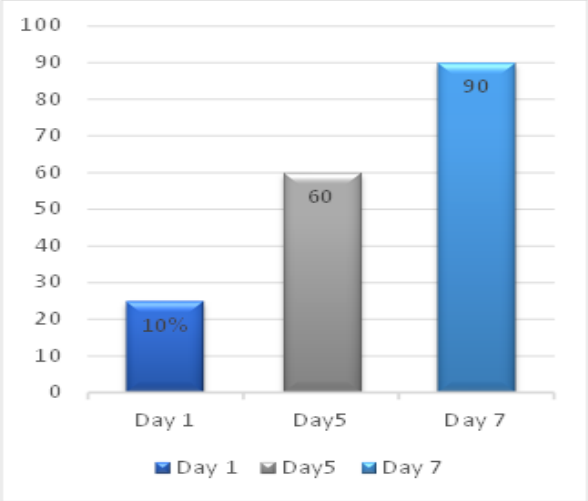


FIG 20: -Percentage of Yeast decolorization of dyes for a different interval of days

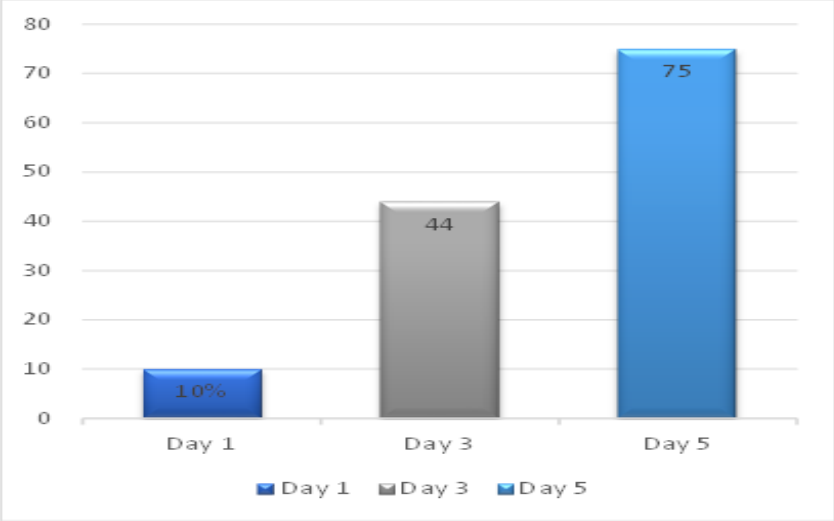


FIG 21: Percentage of Aspergillus Niger decolorization of dyes for a different interval of days

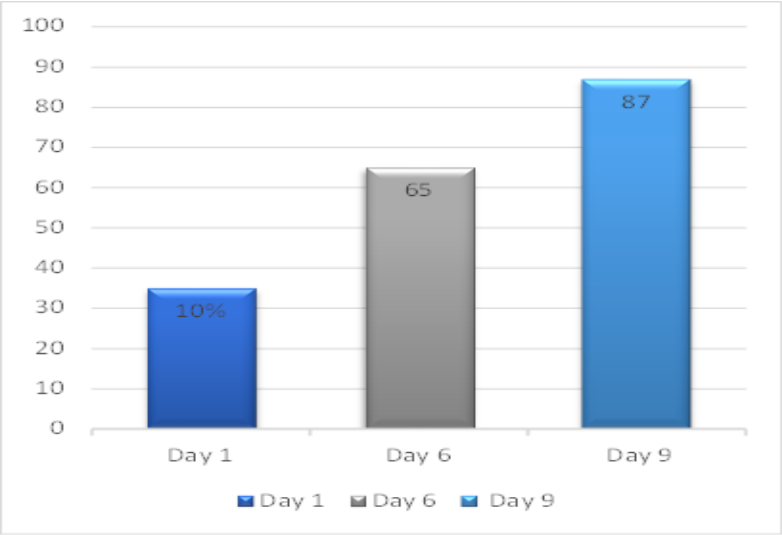


FIG 22: Percentage of Activated charcoal decolorization of dyes for different intervals of time

The figures show the percentage of decolorized clothes over time for different decolorization methods.Figures 19, 20, 21, and 22 show the approximate percentage of discolored clothes over specific time intervals in various methods of decolorization such as bacterial, yeast, fungus, and activated charcoal decolorization.

Observation Table For Dyes

Sr. no	Scientific Name	Family	Plant part used	Color obtained	Extracted coloring pigment
1.	*ClitoriaTernatea*	Solanaceae	Flower	blue	Anthocyanin
2.	*RedMalabarSpinach*	Primulaceae		Dark pink	Anthocyanin
3.	*Bougainvillae*	Nyctaginaceae	flower	Light pink	Anthocyanin
4.	*Impatiensbalsamina*	Balsaminaceae	flower	Buff brown	Xanthophyll
5.	*Mirabilisjalapa*	Nyctaginaceae	flower	Yellowish brown	Anthocyanin
6.	*Turmeric*+ baking soda	Zingiberaceae	roots	Orange	Beta - Carotene
7.	Cestrum nocturnum	Solanaceae	flower	Dark yellow	Xanthophyll

The table depicts the types of pigments that are present in flowers and seeds, which are utilized for dyeing purposes.

Indicates The Intensity

Fabric	Color intensity with mordant		Washing fastness	Rubbing fastness	
	Salt	Cream of Tartar		wet	dry
Cotton	Negative	Positive	Positive	Positive	Positive
Polyester	Negative	Positive	Positive	Positive	Positive

The table above shows how the use of salt and cream of tartar as a mordant affects the intensity of fabric dye absorption.

FIG 23: - Paper Chromatography

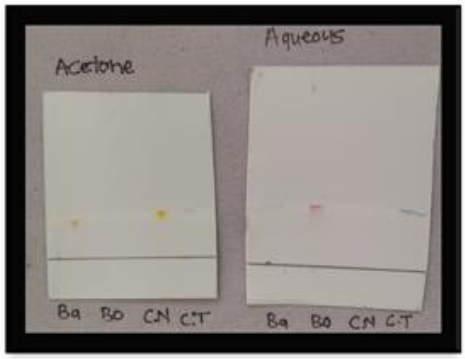


FIG 24: - Thin Layer Chromatography

FIG 25: Chromatography technique

OBSERVATION TABLE FOR PAPER CHROMATOGRAPHY

Pigment	Description of color	Distance Solvent front Traveled	Distance Color Traveled	Rf value	Name of Pigment
1	Blue	4	3.5	0.87	Anthocyanin
2	Light pink	4	3.8	0.95	Anthocyanin
4	Buff brown	4	2.7	0.65	Xanthophyll
5	Yellowish brown	4	2	0.5	Anthocyanin

6	Orange	4	0.3	0.075	Beta- carotene
7	Dark yellow	4	1.6	0.4	Xanthophyll

The table shows the highest Rf value of light pink with Anthocyanin.

Observation Table For Thin layer Chromatography

Sr. no	Flower name	Water	Acetone
1.	Balsamina	0.32	0.20
2.	Bougainvillea	0.34	0.22
3.	Cestrum Nocturnum	0.36	0.22
4.	Clitoria Ternetea	0.34	0.24

Cestrum Nocturnum shows the highest absorbance in water and *Clitoria Ternetea* shows the highest absorbance in acetone as shown in Fig. 25.

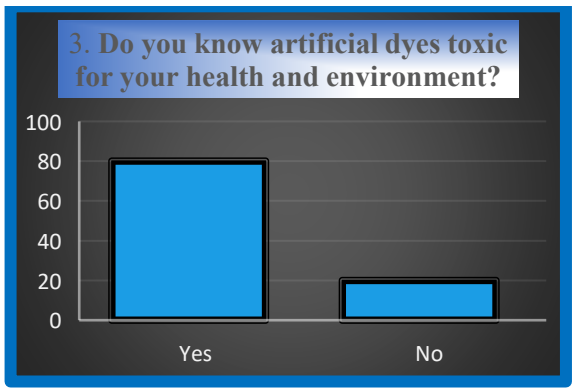
Observation Table For Spectroscopy

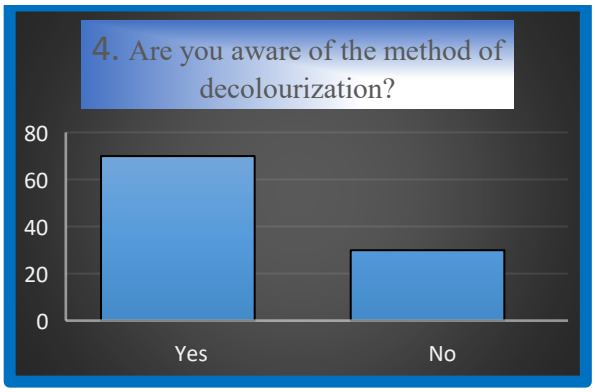
Sr. no	Flower name	Water OD (360 nm)	Isopropanol OD (360 nm)
1.	Bougainvillea	1.73	3.95
2.	Night jasmine	3.37	0.76
4.	Balsamina	3.60	3.70
5.	Turmeric + soda	3	0.98
6.	Mirabilis Jalapa	3.1	3.32

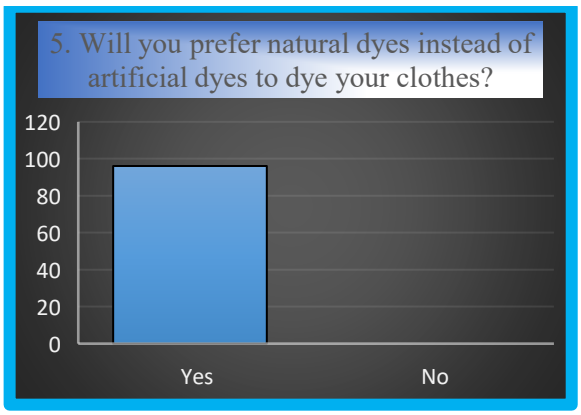
In the table above, it is shown that the Balsamina flower has the highest absorption in water with 3.60 nm while the Bougainvillea flower has the highest absorption in isopropanol with 3.90 nm.

DISCUSSION:

We surveyed Natural Dye and 50 people answered our questions shown as follows:







We conducted a survey to determine people's awareness of the harmful effects of artificial dyes. If people are aware of how hazardous these artificial dyes can be, they may opt for natural dyes made from plant-based pigments instead.

Future Aspect:

The future scope of this study includes the large-scale application of natural dyes in the textile industry and the development of eco-friendly dyeing processes. Natural dyes can also be utilized in products such as paints, crayons, sketch pens, and biodegradable colorants. Further research can focus on improving dye stability, color fastness, and commercial viability. Microbial decolorization techniques may be optimized for industrial wastewater treatment, promoting sustainable textile manufacturing.

CONCLUSION

This study demonstrates that natural dyes obtained from various flowering plants can effectively produce a wide range of colors on fabrics. The use of cream of tartar as a mordant improved dye retention and color intensity. Among the decolorization methods tested, yeast showed the fastest decolorization, followed by bacteria, while fungus and activated charcoal required a longer duration. The findings highlight the potential of natural dyes and microbial decolorizers as sustainable, eco-friendly alternatives to synthetic dyes in the textile industry.

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